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Enhancement of the reactive iron pool by marine diatoms

Micha J.A. Rijkenberg^{a,c,*}, Loes J.A. Gerringa^a, Klaas R. Timmermans^a,
Astrid C. Fischer^b, Koos J. Kroon^b, Anita G.J. Buma^c,
Bert Th. Wolterbeek^b, Hein J.W. de Baar^{a,c}

^a Department of Bio-Chemical Oceanography, Royal Netherlands Institute for Sea Research,
P.O. Box 59, 1790 AB Den Burg, The Netherlands

^b Interfaculty Reactor Institute, Delft University of Technology, Mekelweg 15, 2629 JB Delft, The Netherlands

^c Department of Marine Biology, Centre for Evolutionary and Ecological Studies, University of Groningen,
P.O. Box 14, 9750 AA Haren, The Netherlands

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Abstract

Short term (2 days) laboratory experiments were performed to study the change in irradiance induced production of Fe(II) in seawater in the presence of two open oceanic Southern Ocean diatom species, *Thalassiosira* sp. and *Chaetoceros brevis*. Three irradiance conditions were applied: 1) UVB+UVA+VIS, 2) UVA+VIS, and 3) VIS, and Fe concentrations of 0 and 5 nM Fe were added to natural Southern Ocean seawater (containing 0.32 nM dissolved Fe and 1.69 equivalents of nM L⁻¹ Fe dissolved organic ligands, log K'=22.03). The photoproduced concentration of Fe(II) showed no relationship with the concentration of total dissolved Fe or the concentration of strongly chelated iron. During incubations with the diatoms an increase in the Fe(II) concentration during the second day suggested a modification of the Fe speciation. In the presence of *Thalassiosira* sp. photoreduction of Fe(III) was observed, whereas in the presence of *C. brevis* irradiance independent Fe(III) reduction played an important role in the Fe(II) production. Furthermore, a decrease in the strongly chelated Fe concentration, in concert with a decrease in the conditional stability constant, suggested a modification of the strongly chelated Fe fraction in the experiments with *C. brevis*. The chelated Fe fraction did not change in cultures with *Thalassiosira* sp. Overall, the presence of diatoms appeared to enhance the reactive Fe pool improving the biological availability of Fe.

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1. Introduction

Iron (Fe), present in high concentrations in seawater during the anoxic past of the earth (Turner et al., 2001),

evolved to be a key element in many biochemical reactions, e.g. nitrogen fixation and photosynthesis (Geider et al., 1993). With the ensuing oxygenation of the oceans and the atmosphere, dissolved Fe (DFe) was removed from the oceans and became a “hard to get essential” (Martin and Fitzwater, 1988), inducing the evolution of specialized Fe harvesting strategies in marine organisms (Price and Morel, 1998). In the modern marine environment, the Southern Ocean is an example where Fe, next to light and grazing, is strongly

* Corresponding author. Present address: School of Ocean and Earth Science, University of Southampton, National Oceanography Centre, Southampton SO14 3ZH, United Kingdom. Tel.: +44 2380596237; fax: +44 2380593059.

E-mail address: mzr@noc.soton.ac.uk (M.J.A. Rijkenberg).

limiting primary production (de Baar et al., 2005; de Baar et al., 1990; Martin et al., 1990), thereby affecting biogeochemical cycles: notably that of carbon (de Baar and Boyd, 2000).

The Fe(III) has a very low solubility in seawater and as a consequence hydrolyze rapidly into various Fe(III) oxyhydroxides (Liu and Millero, 2002). The reduced Fe(II), although more soluble in seawater, becomes rapidly oxidized by O₂ and H₂O₂ (King et al., 1995; Millero and Sotolongo, 1989; Millero et al., 1987). Iron solubility is significantly increased by the strong chelation of Fe in the dissolved fraction (<0.2 µm) by organic or inorganic Fe-binding (Boyé et al., 2005; Kuma et al., 1996). These Fe-binding ligands are often found in excess over the DFe pool (Boyé et al., 2001).

Iron redox reactions and speciation are important for the bioavailability of Fe for phytoplankton. Specifically, the Fe redox cycle, as initiated by photochemical processes, is believed to be an important mechanism that converts (colloidal) Fe into more reactive species (defined by the research method applied) resulting in a higher bioavailability for phytoplankton (Finden et al., 1984; Miller and Kester, 1994; Wells and Mayer, 1991). Note, that Fe(II) is suggested to be more bioavailable than Fe(III) (Anderson and Morel, 1980; Anderson and Morel, 1982; Maldonado and Price, 2001; Salmon et al., 2006; Shaked et al., 2005).

The majority of Fe(II) in the marine environment is a result of photo-induced reduction (Croot et al., 2001; Rijkenberg et al., 2005). However, in lakes Fe(II) maxima coincide with chlorophyll-*a* maxima suggesting biological (induced) reduction of Fe(III) (Emmenegger et al., 2001; Shaked et al., 2002). Various phytoplankton species seem capable of enzymatic reduction of Fe(III) at the cell surface (Maldonado and Price, 2000). Recently, it was also suggested that reductase induced dissociative (Shaked et al., 2005) or non-dissociative reduction of Fe bound to Fe-binding ligands (Salmon et al., 2006) might form an important process resulting in Fe uptake and the production of Fe(II) in the surrounding medium. Furthermore, Kuma et al. (1992) observed high concentrations of Fe(II) during spring blooms in Funka Bay (Japan) and related this to the release of organic components from phytoplankton inducing the photoreduction of Fe. The presence of various dissolved organic substances, generally considered to be released by phytoplankton, e.g. sugar acids, indeed improved the photoreduction of Fe (Hudson et al., 1992; Kuma et al., 1995; Öztürk et al., 2004; Song et al., 2005).

There are marine Fe(III)-siderophore complexes, which are high affinity Fe(III) binding ligands excret-

ed by e.g. marine bacteria to scavenge and transport Fe (Granger and Price, 1999; Trick, 1989; Wilhelm and Trick, 1994), that are photo-reactive (Barbeau, 2006; Barbeau et al., 2001). Photoredox reactions of Fe-ligand complexes occur via a ligand to metal charge transfer (LMCT) reaction resulting in the production of Fe(II) and an irreversible decomposed organic ligand (Waite and Morel, 1984). If the ligand production is less than the photodegradation, the concentration of photo-reactive Fe-binding ligands decreases under irradiance, thus leaving behind non-photoreactive Fe-binding ligands (Boyé et al., 2001; Powell and Wilson-Finelli, 2003). Yet, the importance of siderophores or other strong Fe-binding ligands in the photoproduction of Fe(II) in the marine environment is still uncertain (Rijkenberg et al., 2006b).

In this study we investigated the influence of two typical open oceanic Southern Ocean diatom species, *Thalassiosira* sp. and *Chaetoceros brevis*, on the photo-induced changes in Fe speciation in Southern Ocean seawater. We described three experiments where the phytoplankton species, the Fe concentration and irradiance were varied. Short term experiments were conducted to allow observation of an immediate, or at least short-term, response of Fe speciation while the plankton community remained relatively uniform (Timmermans et al., 1998).

2. Materials and methods

2.1. Diatoms

C. brevis is a small (4–6 µm) single celled diatom, with a growth response in relation to the availability of Fe (K_m) of 0.6×10^{-3} nmol L⁻¹ and a maximal growth rate of 0.39 d⁻¹ (Timmermans et al., 2001). *Thalassiosira* sp. is a large (70 µm) chain forming diatom, with a K_m of 0.62 nmol L⁻¹ and a maximal growth rate of 0.31 d⁻¹ (Timmermans et al., 2004). Both strains were isolated from the Southern Ocean, *Thalassiosira* sp. by Philipp Assmy (Alfred Wegener Institut für Polar und Meeresforschung in Bremerhaven, Germany) and *C. brevis* by Anita Buma (Rijksuniversiteit Groningen, The Netherlands) and kept in culture by Klaas Timmermans (Royal Netherlands Institute for Sea Research, The Netherlands) (Timmermans et al., 2001; Timmermans et al., 2004). *C. brevis* and *Thalassiosira* sp. cells were pre-cultivated in the same Southern Ocean seawater with the same temperature (4 °C) and day/night cycle (16 h:8 h respectively) as used during the experiments, however with a 5 nmol L⁻¹ Fe addition, in order to generate sufficient biomass for the experiments.

Prior to the experiments *Thalassiosira* sp. cells were taken from Southern Ocean seawater with silicon tubing as follows:

one opening of the silicon tubing was covered with a fine acid-cleaned $\sim 36 \mu\text{m}$ polyamide (ZBF, Mesh+Technology, Switzerland) filter. The culture was then gently sucked through the filter with a vacuum pump. Cells were subsequently rinsed and gently resuspended in Southern Ocean seawater. This procedure was followed to concentrate the cells. The cells were counted, followed by inoculation in the experimental UV transparent polymethylmetacrylate (PMMA, see below) bottles. The density of *Thalassiosira* sp. was about 50 ± 12 cells/mL at $t=0$ in the incubations. The *C. brevis* culture was dense enough to inoculate the PMMA bottles directly. The density of *C. brevis* was about $1.04 \pm 0.08 \cdot 10^4$ cells/mL at $t=0$ in the incubations.

Thalassiosira sp. cells were counted in 5 mL settling chambers under an inverted microscope; *C. brevis* was counted using an Epics XL flowcytometer.

2.2. Seawater

Clean Southern Ocean surface water (collected 51°S 20°E , 1 November 2000) was collected from a torpedo towed at approximately 5 m alongside the ship (Polarstern, ANT XVIII/2) and via acid-washed braided PVC tubing pumped into an over-pressurized class 100 clean air container using a Teflon diaphragm pump (Almatec A-15, Germany) driven by a compressor (Jun-Air, Denmark, model 600-4B). The seawater was filtered in-line by a filter (Sartorius Sartobran filter capsule 5231307H8) with a cut-off at $0.2 \mu\text{m}$ and then led into a large tank (volume of 1 m^3). Upon return to the home laboratory the tank was stored at room temperature. The Southern Ocean seawater for the experiments contained at the time that we used it for the experiments 0.32 nmol L^{-1} DFe and 1.69 ± 0.19 equivalents of nmol L^{-1} of Fe of Fe-binding ligand with a conditional stability constant ($\log K'$) of 22.03 ± 0.24 (given errors show the 95% confidence interval). Nutrient concentrations were: $26 \mu\text{mol L}^{-1}$ nitrate, $24.45 \mu\text{mol L}^{-1}$ silicate, $1.82 \mu\text{mol L}^{-1}$ phosphate.

2.3. Irradiance treatments

Philips UVB (TL-12), UVA (TL' 40W/05) and VIS (TL'D 36W/33) lamps were used in combination with Mylar, 50% cut-off at 320 nm, and UV-opaque PMMA filter, 50% cut-off at 380 nm, to create the three different optical treatments: UVB+UVA+VIS, UVA+VIS, and VIS in the UV-transparent PMMA bottles (50% cut-off at 290 nm) (Rijkenberg et al., 2005; Steeneken et al., 1995). Spectral conditions were measured inside each bottle using a MACAM Spectroradiometer SR9910 with a small spherical 4π sensor. Spectra were recorded from 280–700 nm. The two spectra of the duplicate bottles of the UVB+UVA+VIS and VIS optical conditions were very similar. The two spectra of the duplicate bottles receiving UVA+VIS showed an intensity difference (Fig. 1). Integrated values of the irradiance conditions for UVB, UVA and VIS as measured inside the bottles are given in Table 1.

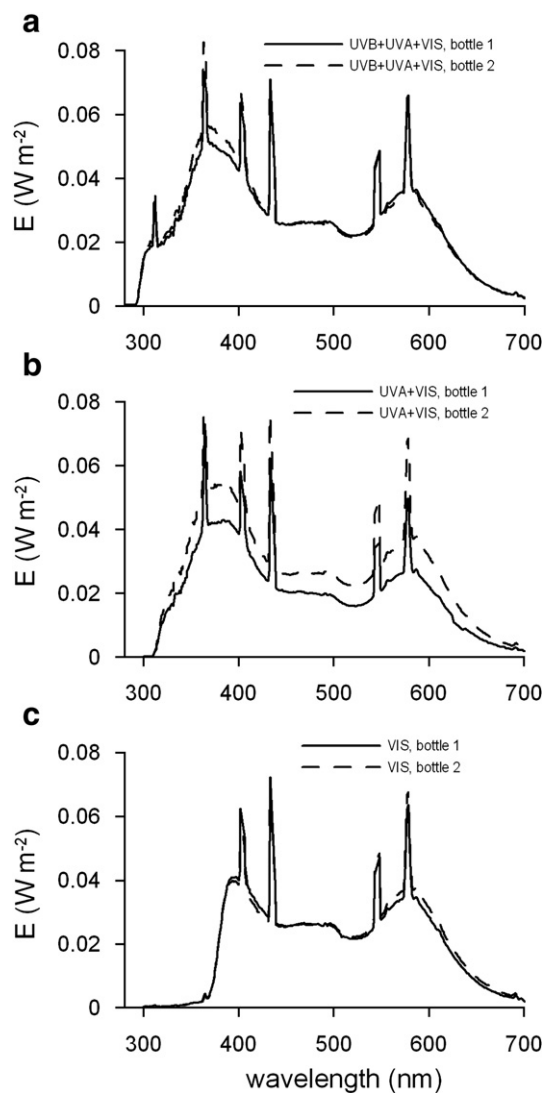


Fig. 1. The light spectra as measured in the PMMA bottles receiving UVB+UVA+VIS (a), UVA+VIS (b) and VIS (c).

2.4. Experimental

The experiments were performed in a temperature controlled class 100 clean container. The temperature was kept at 4°C . A $20 \mu\text{mol L}^{-1}$ Fe(III) stock solution was made with ammonium Fe(III) sulfate ($\text{NH}_4\text{Fe(III)(SO}_4)_2 \cdot 12\text{H}_2\text{O}$, Baker Analyzed, reagent grade) in 0.012 mol L^{-1} three times quartz distilled ($3 \times \text{QD}$) HCl using $18 \text{ M}\Omega$ nanopure water.

Three experiments were performed: experiment A with *Thalassiosira* sp. (starting with 50 cells mL^{-1}) and an addition of 5 nmol L^{-1} Fe(III), experiment B with *Thalassiosira* sp. (starting with 50 cells mL^{-1}) without Fe(III) addition and experiment C with *C. brevis* (starting with $10,500 \text{ cells mL}^{-1}$) and an addition of 5 nmol L^{-1} Fe(III). The walls of the 2 L PMMA bottles were equilibrated prior to use with Southern Ocean seawater containing the same Fe(III) concentration as

Table 1

Integrated values of UVB, UVA and VIS irradiance as measured within the PMMA bottles receiving different irradiance treatments for all three experiments

	UVB+UVA+VIS		UVA+VIS		VIS	
	Bottle 1 ^a	Bottle 2	Bottle 1	Bottle 2	Bottle 1	Bottle 2
UVB (Wm ⁻²)	0.38	0.41	0.02	0.02	0.01	0.01
UVA (Wm ⁻²)	3.45	3.77	2.77	3.52	0.98	0.96
VIS (Wm ⁻²)	8.71	8.61	6.75	8.98	8.52	8.71

^a To give an idea of the irradiance flux used in these experiments the UVB flux in bottle 1 receiving UVB+UVA+VIS compares with the UVB flux at 18 m depth, the UVA flux in this bottle compares with the UVA flux at 59 m depth and the VIS flux in this bottles compares with the VIS flux at 85 m depth in the Southern Ocean near Palmer station (irradiance spectrum 9 Nov 2000 16:00 UTC, attenuation coefficients as determined by (Boucher and Prezelin, 1996)).

during the experiment. Addition of 5 nmol L⁻¹ Fe(III) in experiments A and C resulted in the formation of amorphous ferric oxyhydroxides after a 12 h equilibration period (Rijkenberg et al., 2006a). Each experiment started with the addition of an equal amount of algae to 7 PMMA bottles containing Southern Ocean seawater. One bottle (*t*=0) was immediately sampled for DFe, Fe ligands and cell numbers. The other bottles (2 bottles for each light condition) were sub-sampled during the experiments. At regular intervals the bottles were gently shaken.

Samples for DFe and FeL were gently filtered inside a flow bench using acid cleaned 0.22 µm polycarbonate filters (Micron Separations Inc.) in an acid-cleaned filter holder (Nalgene) connected to a vacuum pump. The Fe(II) concentration was immediately determined via inline sampling (see below).

The concentration of Fe(II) was also measured in an abiotic experiment using the same seawater and an addition of 3 nM Fe(III) but without diatoms. This abiotic experiment was performed on a separate occasion under UVB+UVA+VIS irradiance with different intensities as used during the experiments with the diatoms. The goal was to investigate the qualitative behaviour of the Fe(II) concentration with time in the absence of diatoms.

2.5. Iron(II) analysis

Concentrations of Fe(II) were regularly measured inline via an acid cleaned 0.2 µm acrodisc filter (refreshed each day) using an automated flow injection analysis system employing a luminol-based chemiluminescence detection of Fe(II) (Croot and Laan, 2002; King et al., 1995; Seitz and Hercules, 1972). An alkaline luminol solution was mixed with the sample in a spiral shaped flow cell in front of a Hamamatsu HC135 photon counter. At pH 10, Fe(II) is rapidly oxidized by oxygen on a millisecond time scale causing the oxidation of luminol producing blue light (Xiao et al., 2002). Samples were transported in-line from the PMMA bottle to a sample loop. Then the sample was, by introducing it into 18 MΩ cm⁻¹ nanopure water (MQ-water, Millipore), transported into the flow cell every 93 s. The complete analytical system was built in a light-tight wooden box. The luminol reagent and the carrier were kept in light tight bags (as used for storage of

photographic films). The tubing was covered with aluminum foil and the tubing of the peristaltic pump was shaded by black plastic.

The alkaline luminol solution was prepared weekly with 15 mmol L⁻¹ 5-amino-2,3-dihydro-1,4-phthal-azinedione (Luminol) (SIGMA) in 20 mmol L⁻¹ Na₂CO₃. The 50 µmol L⁻¹ luminol reagent solution was made in 0.5 mol L⁻¹ NH₃ (suprapur, Merck) and 0.1 mol L⁻¹ HCl (suprapur, Merck). The luminol reagent solution was stored in the dark for at least 24 h before use, to ensure that the reagent properties had stabilized. An 0.01 mol L⁻¹ Fe(II) stock was prepared monthly by dissolving ferrous ammonium sulfate hexahydrate (Fe(II) (NH₄SO₄)₂6H₂O) (Baker Analyzed, reagent grade) in 0.012 mol L⁻¹ (3 × QD) HCl. Working solutions were prepared daily. All Fe(II) stock solutions were kept in the dark at 4 °C when not in use.

Calibration was performed once a day by 6 standard additions of known Fe(II) concentrations (between 0.001 and 4 nM Fe(II)) to a separate batch of the same Southern Ocean seawater. The chemiluminescence signal responded in a quadratic way to the Fe(II) concentration (Moffet et al., 2007; Rose and Waite, 2001). The time delay between Fe(II) addition and measurement caused an oxidation effect. This oxidation effect was accounted for by extrapolating the data back to time zero using the fact that the oxidation of Fe(II) in seawater very closely approximates pseudo-first-order kinetics.

The detection limit, calculated as 3σ of the steady state Fe(II) concentration in the dark using the experimental seawater (4 °C), was on average 8.3 ± 3 pM as determined on 12 separate occasions. The steady state Fe(II) values and standard errors are based on a mathematical fit of between 9 and 45 datapoints.

Rose and Waite (2001) found a relationship between the interference of superoxide and the concentration of Fe(II) being measured. Using a model they calculate that the concentration of superoxide in the sample is smaller than 1% of the superoxide generated in the flow cell. Furthermore, they find that beneath 32 nM Fe(II) the relationship between the CL signal and Fe(II) is unique. Additionally, in general, open ocean seawater contains very low concentrations of CDOM (α₃₇₅ of 0.05–0.3 m⁻¹) (Bricaud et al., 1981). Indeed, seawater that we sampled during the same cruise in the Southern Ocean contained a very low CDOM concentration

with an α_{375} of 0.05 m^{-1} (Rijkenberg et al., 2005). Low concentrations of CDOM would limit the production of superoxide (Micinski et al., 1993) so that superoxide production would not interfere with the measurement of Fe(II) (Laglera and Van den Berg, 2007).

2.6. Analysis of total DFe

DFe, defined as the Fe fraction passing a $0.2 \mu\text{m}$ filter, was determined using flow injection analysis with luminol chemiluminescence and H_2O_2 (de Jong et al., 1998).

2.7. CLE-ACSV

Determination of the organic speciation of Fe in seawater water was performed using competitive ligand exchange-adsorptive cathodic stripping voltammetry (CLE-ACSV). The reagent 2-(2-Thiazolylazo)-*p*-cresol (TAC) (Aldrich, used as received) was used as competing ligand (Croot and Johansson, 2000). All solutions were prepared using $18.2 \text{ M}\Omega$ nanopure water. The equipment consisted of a $\mu\text{Autolab}$ voltammeter (Ecochemie, Netherlands), a static mercury drop electrode (Metrohm Model VA663), a double-junction Ag/saturated AgCl reference electrode with a salt bridge containing 3 mol L^{-1} KCl and a counter electrode of glassy carbon. Standard solutions were as follows: a 0.01 mol L^{-1} stock solution of TAC in $3 \times \text{QD}$ methanol, a 1 mol L^{-1} boric acid (Suprapur, Merck) in 0.3 mol L^{-1} ammonia (Suprapur, Merck) to buffer the samples to a pH of 8.05 and a $10^{-6} \text{ mol L}^{-1}$ Fe(III) stock solution acidified with 0.012 mol L^{-1} HCl ($3 \times \text{QD}$). Fe was removed from the mixed $\text{NH}_3/\text{NH}_4\text{OH}$ borate buffer by the addition of TAC and subsequent binding of TAC and $\text{Fe}(\text{TAC})_2$ on a C_{18} SepPak column. Aliquots of 15 mL were spiked with Fe(III) with final concentrations between 0 and 20 nmol L^{-1}

and allowed to equilibrate overnight ($>15 \text{ h}$) with 5 mmol L^{-1} borate buffer and $10 \mu\text{mol L}^{-1}$ TAC. The concentration $\text{Fe}(\text{TAC})_2$ in the samples were measured using the following procedure: i) removal of oxygen from the samples for 200 s with dry nitrogen gas, a fresh Hg drop was formed at the end of the purging step, ii) a deposition potential of -0.40 V was applied for $30\text{--}60 \text{ s}$ depending on the sample measured the solution was stirred to facilitate the adsorption of the $\text{Fe}(\text{TAC})_2$ to the Hg drop, iii) at the end of the adsorption period the stirrer was stopped and the potential was scanned using the differential pulse method from -0.40 to -0.90 V at 19.5 mV s^{-1} and the stripping current from the adsorbed $\text{Fe}(\text{TAC})_2$ recorded.

The ligand concentration and the conditional stability constant were calculated using the non-linear fit of the Langmuir isotherm (Gerringa et al., 1995).

3. Results

3.1. Diatoms

The average growth rate of *Thalassiosira* sp. was $0.41 \pm 0.22 \text{ d}^{-1}$ (1σ , $n=6$) in experiment A (5 nmol L^{-1} Fe(III) addition) and $0.26 \pm 0.28 \text{ d}^{-1}$ (1σ , $n=6$) in experiment B (no Fe(III) added). The average growth rate of *C. brevis* was $0.34 \pm 0.10 \text{ d}^{-1}$ (1σ , $n=5$) (5 nmol L^{-1} Fe(III) addition) in experiment C.

The diatom cultures used during these experiments were not axenic. However, bacterial counts in other experiments with *C. brevis* were low (Timmermans, K.R. pers. comm.). Most bacteria in the *Thalassiosira* sp. culture were removed after the filtration, concentration and subsequent resuspension of *Thalassiosira* sp. in the seawater for the experiments. Therefore bacteria are ignored in the below interpretation of the Fe chemistry.

Table 2

The concentrations DFe (nmol L^{-1}) for the experiments A ($+5 \text{ nmol L}^{-1}$ Fe and *Thalassiosira* sp.), B (*Thalassiosira* sp.) and C ($+5 \text{ nmol L}^{-1}$ Fe and *C. brevis*.)

Exp./time	DFe (nmol L^{-1})	UVB+UVA+VIS DFe (nmol L^{-1})		UVA+VIS DFe (nmol L^{-1})		VIS DFe (nmol L^{-1})	
		Bottle 1	Bottle 2	Bottle 1	Bottle 2	Bottle 1	Bottle 2
A/ $t=0$	3.74 ± 0.19	—	—	—	—	—	—
A/Day 1 end	—	1.94 ± 0.06	3.85 ± 0.01	3.14 ± 0.05	5.6 ± 0.04^a	1.6 ± 0.12	3.94 ± 0.08
A/Day 2 begin	—	1.92 ± 1.1	0.98 ± 0.01	2.28 ± 0.12	1.3 ± 0.02	1.21 ± 0.04	2.68 ± 0.06
A/Day 2 end	—	2.91 ± 0.25	1.4 ± 0.04	2.27 ± 0.06	1.5 ± 0.31	1.64 ± 0.04	2.26 ± 0.09
B/ $t=0$	0.4 ± 0.02	—	—	—	—	—	—
B/Day 1 end	—	0.28 ± 0.02	0.25 ± 0.02	0.71 ± 0.11^a	0.37 ± 0.002	0.19 ± 0.01	0.22 ± 0.003
B/Day 2 begin	—	0.32 ± 0.01	0.62 ± 0.05	0.31 ± 0.02	0.54 ± 0.03	0.25 ± 0.03	0.4 ± 0.03
B/Day 2 end	—	0.2 ± 0.02	0.18 ± 0.001	0.3 ± 0.02	0.08 ± 0.003	0.28 ± 0.06	0.33 ± 0.04
C/ $t=0$	3.71 ± 0.03	—	—	—	—	—	—
C/Day 1 end	—	2.23 ± 0.01	—	1.68 ± 0.17	2.34 ± 0.02	1.67 ± 0.01	2.26 ± 0.06
C/Day 2 begin	—	2.42 ± 0.62	2.48 ± 0.07	1.67 ± 0.01	1.64 ± 0.02	2.09 ± 0.01	2.15 ± 0.02
C/Day 2 end	—	1.31 ± 0.01	1.9 ± 0.01	1.2 ± 0.01	1.42 ± 0.01	1.43 ± 0.05	2.7 ± 0.05

Samples were taken directly after the addition of the phytoplankton for $t=0$. The errors give the standard deviation (stdev) of the analysis ($n=2$).

^a These values are higher than expected, possibly due to contamination.

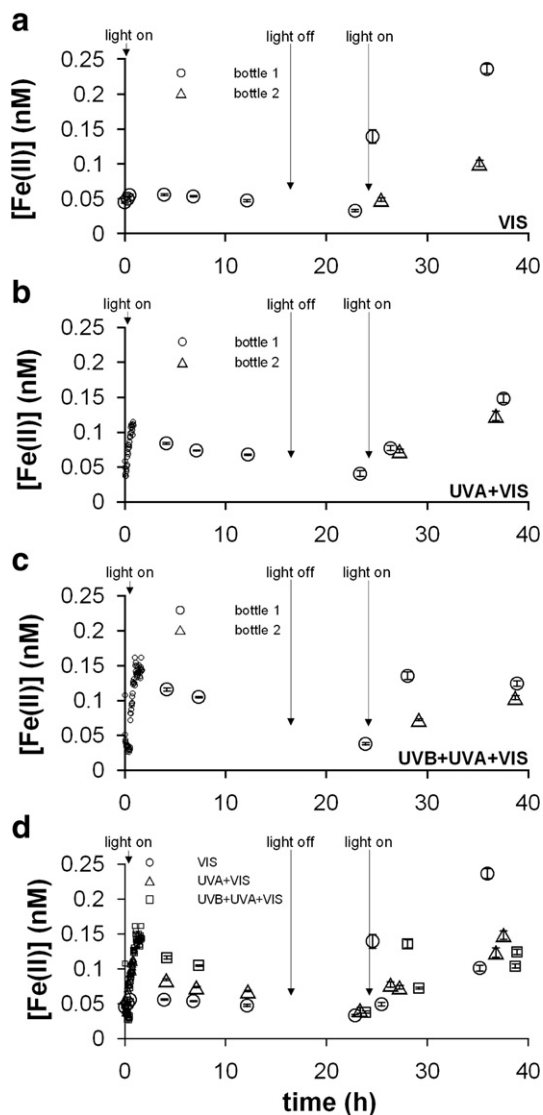


Fig. 2. The Fe(II) concentration (nM) in experiment A (addition of 5 nM Fe(III) and *Thalassiosira* sp.) receiving a) VIS in bottles 1 and 2, b) UVA + VIS in bottles 1 and 2, and c) UVB + UVA + VIS in bottles 1 and 2. Figure d) shows the Fe(II) concentration for the bottles 1 and 2 in response to: VIS, UVA + VIS and UVB + UVA + VIS. The larger symbols give the steady state values and standard errors based on a mathematical fit of between 9 and 45 datapoints. The small symbols in the graphs show individual data points of the Fe(II) concentrations direct after turning on the light. The detection limit of Fe(II) was on average 9.4 ± 0.4 pM.

3.2. Total DFe

The Southern Ocean seawater contained 0.32 nmol L^{-1} total DFe and 1.69 equivalents of nmol L^{-1} Fe (eq nmol L^{-1} Fe) ligand concentration, i.e. with an excess ligand concentration of $1.37 \text{ eq nmol L}^{-1}$ Fe (excess L). Upon addition of 5 nmol L^{-1} Fe(III), it was assumed that at maximum 1.37 nmol

L^{-1} of the added Fe was complexed by ligands in the dissolved fraction, and that approximately 3.63 nmol L^{-1} Fe(III) could precipitate within 1 to 2 h as inorganic Fe colloids (Rijkenberg et al., 2006a). After addition of the diatoms a DFe concentration of $\sim 3.7 \text{ nmol L}^{-1}$ in experiments A and C was measured (Table 2). This means that 1.6 nmol L^{-1} (5.32 nmol L^{-1} DFe– 3.7 nmol L^{-1} DFe) was either present as fresh Fe precipitates, $>0.2 \mu\text{m}$, or adsorbed by either diatoms or the bottle wall. A further $\sim 50\%$ decrease of DFe was observed at the end of the experiments. When assuming that

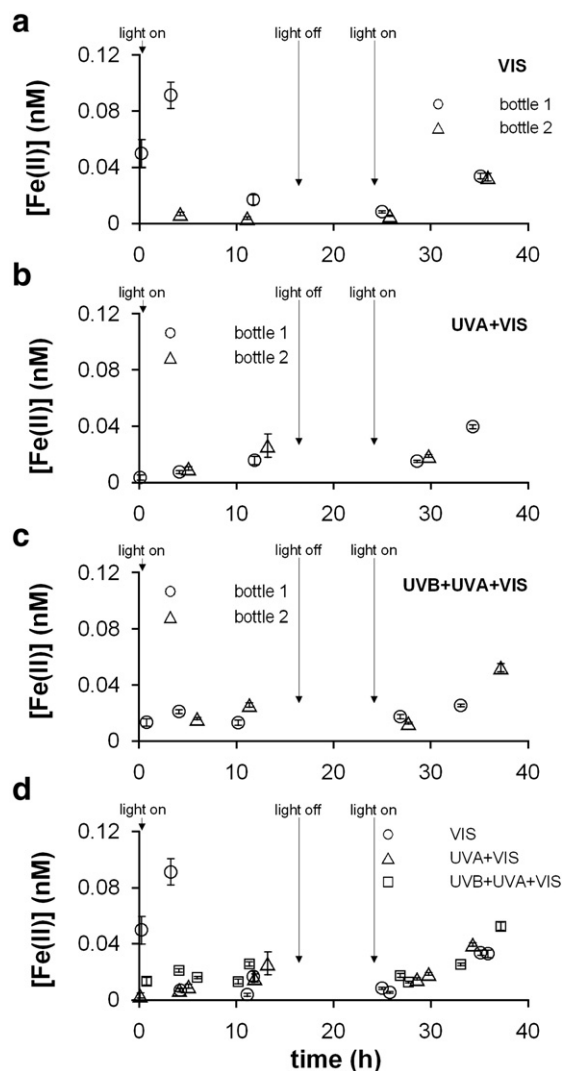


Fig. 3. The Fe(II) concentration (nM) during experiment B (in the presence of *Thalassiosira* sp., no addition of Fe) receiving a) VIS in bottles 1 and 2, b) UVA + VIS in bottles 1 and 2, and c) UVB + UVA + VIS in bottles 1 and 2. Figure d) shows the Fe(II) concentration for the bottles 1 and 2 in response to: VIS, UVA + VIS and UVB + UVA + VIS. The larger symbols give the steady state values and standard errors based on a mathematical fit of between 9 and 45 data points. The detection limit of Fe(II) was on average 9.4 ± 2.7 pM.

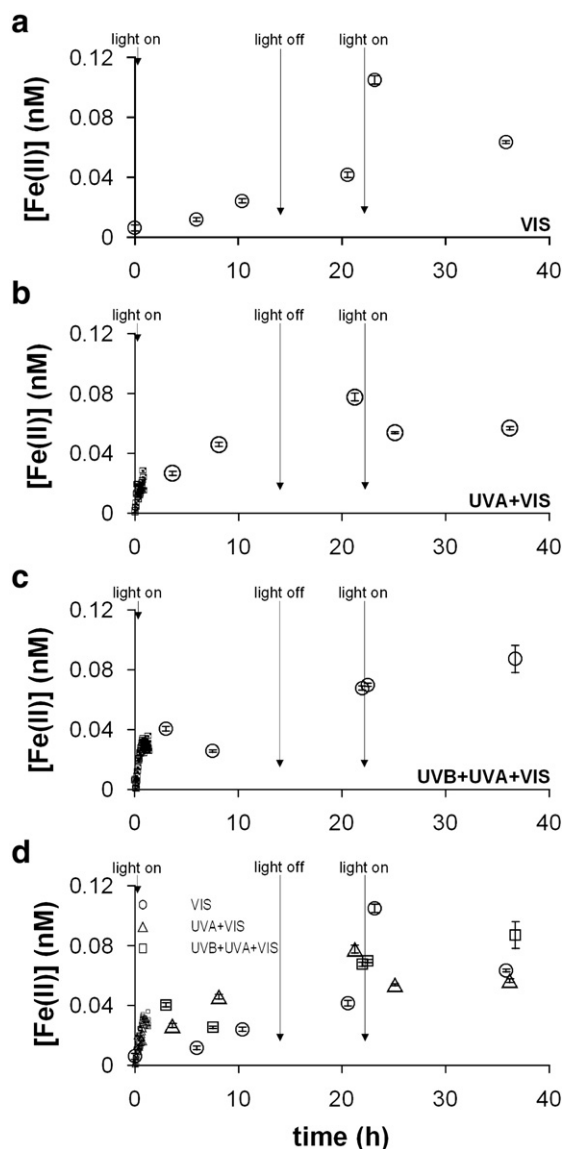


Fig. 4. The Fe(II) concentration (nM) in experiment C (in the presence of *C. brevis*, addition of 5 nmol L⁻¹ Fe(III)) receiving a) VIS, b) UVA + VIS, and c) UVB + UVA + VIS. Figure d) shows the Fe(II) concentration for all three light treatments (VIS, UVA + VIS and UVB + UVA + VIS). The larger symbols give the steady state values and standard errors based on a mathematical fit of between 9 and 45 data points. The small symbols in the graphs show individual data points of the Fe(II) concentrations direct after turning on the light. The detection limit of Fe(II) was on average 5.0 ± 1.9 pM.

the average concentration of DFe only decreased due to uptake and adsorption by the diatoms, we calculated the uptake/adsorption rates for *Thalassiosira* sp. in experiment A as $9.53 \cdot 10^{-16}$ mol Fe cell⁻¹ h⁻¹, in experiment B as $1.77 \cdot 10^{-16}$ mol Fe cell⁻¹ h⁻¹ and for *C. brevis* in experiment C as $7.8 \cdot 10^{-18}$ mol Fe cell⁻¹ h⁻¹. Hudson and Morel (1990) found uptake rates in Fe depleted *Thalassiosira weissflogii* in the order of 10^{-16} –

10^{-18} mol Fe cell⁻¹ h⁻¹. The literature values agree well with the values obtained in our experiments. Furthermore, adsorption of Fe to the PMMA bottle wall would be small after 36 h equilibration preceding the addition of the diatoms (Fischer et al., 2007). We cannot quantify other possible sinks of DFe like the formation of Fe colloids in our experiments.

In experiment B the DFe concentration was lower than the total ligand concentration (Table 2). Theoretically all Fe in experiment B was complexed by Fe-binding ligands but, as observed before, non-negligible amounts of amorphous Fe oxyhydroxides might persist in spite of the presence of organic ligands with high conditional stability constants (Rijkenberg et al., 2006a).

The concentrations of DFe as function of the light treatments were too variable to draw conclusions with regard to co-variance between Fe(II) and DFe.

3.3. Ferrous iron

The Fe(II) concentration increased from the first to the second experimental day in all three experiments (Figs. 2–4). In most experiments the Fe(II) concentration increased up to 8–12% of the DFe concentration. Note that the relatively high Fe(II) concentrations were a result of the total Fe concentration, the light climate and the slow back oxidation rates at 4 °C (Kuma et al., 1995). The net Fe(II) production rates are given in Table 3.

3.3.1. Day 1, lights on

In the abiotic experiment with UVB + UVA + VIS irradiance, the Fe(II) concentration of the Southern Ocean seawater, without diatoms, showed a steep increase when turning on the light (Fig. 5). After a maximum in the Fe(II) concentration was attained, the Fe(II) concentration decreased towards a steady state over time. The same parabolic pattern of Fe(II) concentration was also observed in the experiments A and C (both +5 nM Fe(III)). This process has also repeatedly been observed after addition of Fe(III) to seawater, forming mixed inorganic/organic colloidal Fe, in other experiments (Rijkenberg et al., 2006a; Waite and Morel, 1984), and even induced by pre-irradiating water and thus destroying all organic substances (Emmenegger et al., 2001). A decrease in Fe(II) concentration during irradiance can be explained by two processes: an increase in oxidation of Fe(II) (Rijkenberg et al., 2006a) or a decrease in the amount of photoreducible Fe due to the modification of the colloidal Fe surface by photochemical processes (Wells and Mayer, 1991). Note that the abiotic experiment was performed on a separate occasion under different irradiance intensities so the absolute Fe(II) concentration cannot be compared with the Fe(II) concentrations in the incubations with diatoms.

Photoproduced Fe(II) concentrations are strongly dependent on the intensity and wavelength of an irradiance treatment (Laglera and Van den Berg, 2007; Rijkenberg et al., 2005; Rijkenberg et al., 2004). In experiment A (*Thalassiosira* sp., +5 nM Fe(III) addition), the concentration of Fe(II) increased with irradiance treatment where: UVB + UVA + VIS > UVA +

Table 3

The initial Fe(II) production rates at $t=0$ (nM/s) and the net Fe(II) production rates (nM/s) assuming constant oxidation for the experiments A, B and C

(x 10 ⁻⁶ nM/s)	VIS		UVA + VIS		UVB + UVA + VIS	
	Bottle 1	Bottle 2	Bottle 1	Bottle 2	Bottle 1	Bottle 2
<i>Experiment A</i>						
$t=0$	–	–	65.3	–	147	–
$t=0$ — Fe(II) max	5.72	–	36.5	–	41.7	–
Fe(II) max — dark	–0.31	–	–1900	–	–1.97	–
Second day	2.36	1.47	1.75	1.47	–0.28	0.92
<i>Experiment B</i>						
$t=0$	–	–	–	–	372	–
$t=0$ — Fe(II) max	3.83	–	–	–	0.64	–
Fe(II) max — dark	–2.42	–0.14 ^a	0.29 ^a	0.56 ^a	–0.36	0.50 ^a
Second day	0.69	0.75	1.19	–	0.36	1.17
<i>Experiment C</i>						
$t=0$	–	–	22.6	–	29.3	–
$t=0$ — Fe(II) max	–	–	6.22	–	12.9	–
Fe(II) max — dark	0.47 ^a	–	0.67	–	–0.91	–
Second day	–0.91	–	0.071	–	0.34	–

The net Fe(II) production rates were determined for 3 periods during the experiments. These periods were: i) between $t=0$ and the first Fe(II) concentration maximum (Fe(II) max), ii) between the first Fe(II) concentration maximum and the dark period, and iii) during the second day. When oxidation exceeds formation the net Fe(II) production has a negative sign.

^a As no Fe(II) concentration maximum was measured the value gives the net Fe(II) production rate during the first day.

VIS>VIS on the first day. But on the second day, an increase with irradiance treatment was less clear (Fig. 2). During experiment B (*Thalassiosira* sp., no Fe(III) addition) (Fig. 3), low concentrations of DFe (~ 0.32 nmol L⁻¹) and very low

concentrations of Fe(II) were measured at the first day of the experiment (~ 20 pmol L⁻¹ Fe(II), Fig. 3). Furthermore, no wavelength effect was observed in the Fe(II) concentration i.e. UVA and UVB did not play a major role in Fe(II) production

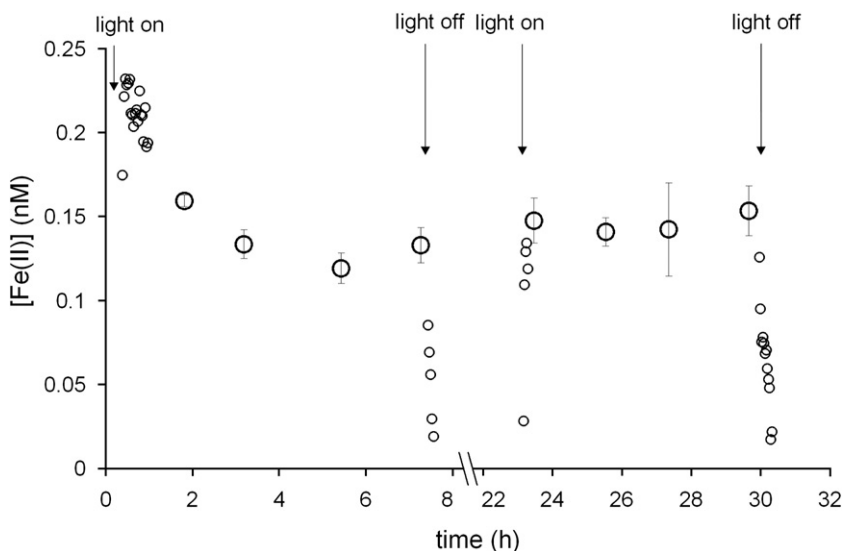


Fig. 5. The abiotic experiment, without diatoms, shows the concentration of Fe(II) (nmol L⁻¹) when an UVB+UVA+VIS irradiance treatment was used over a period of 2 days with an 3 nM Fe(III) addition to seawater. The change with time during the first day shows the typical rapid increase until a maximum Fe(II) concentration is attained, and is followed by a decrease in Fe(II) concentration during the light period until a steady state is approached. The concentration of Fe(II) on the second day is similar to the concentration of the approached steady state during the first day. The error bars give the standard error. The small symbols in the graphs show individual data points of Fe(II) concentrations.

(Fig. 3d). The lack of a clear wavelength effect on the Fe(II) concentrations might be due to very low Fe(II) concentrations, or because the Fe(III) might be partly complexed by photostable Fe-binding ligands. During experiment C (*C. brevis* + 5 nmol L⁻¹ DFe), the Fe(II) concentrations increased with irradiance treatment as in experiment A (Fig. 4). In the bottle receiving VIS, the Fe(II) concentration increased from the start of the experiment and did not reach a maximum, as discerned from the modest number of data points (Fig. 4a). The Fe(II) concentration in the bottle receiving UVA+VIS decreased after reaching a maximum, followed by an additional increase at 10 h (Fig. 4b). The Fe(II) concentration in the bottle receiving UVB+UVA+VIS decreased after reaching the Fe(II) maximum during day 1 (Fig. 4c).

3.3.2. Day 1, lights off

The Fe(II) concentration in the abiotic experiment (data not shown) and experiment A returned to near pre-irradiance concentrations in the dark. The Fe(II) concentration at the end of the dark period in experiment C was higher than the Fe(II) concentration during irradiance on the previous day. These high Fe(II) concentrations in the dark may be ascribed to two different conceivable mechanisms: reduction of Fe(III) in the dark by either superoxide produced by *C. brevis* as was shown by Kustka et al. (2005) for the diatoms *Thalassiosira weissflogii* and *Thalassiosira pseudonana* or by extra-cellular enzymes (Maldonado and Price, 2000; Salmon et al., 2006; Shaked et al., 2005).

3.3.3. Day 2, lights on

The concentration of Fe(II) increased during the second day in the experiments A and B, and was greater than concentrations measured on the first day (Figs. 2, 3). However, no difference in Fe(II) concentration was observed due to the wavelength range of the irradiance treatments. In contrast, on the second day, the abiotic experiment receiving UVB+UVA+VIS irradiance did not show an increase but instead a steady state in the Fe(II) concentration (Fig. 5). The Fe(II) concentrations in experiment C did not show such an uniform response (Fig. 4). The Fe(II) concentration increased only in the bottle receiving UVB+UVA+VIS. The Fe(II) concentration in the bottle receiving VIS decreased, whereas it appeared to have reached a steady state in the bottle receiving UVA+VIS. Overall, the increase in the concentration of Fe(II) at the second day in experiment A and B and the high concentrations of Fe(II) during the dark in experiment C imply that the concentrations of Fe(II) increased as a result of the presence of the diatoms.

3.4. Organically complexed ferric iron

After introduction of the algae in experiment A, B and C strong Fe-binding ligands (L) were present with an average of the logarithm of the conditional stability constants (log *K'*) of: 22.15±0.16 (*n*=6), 21.73±0.13 (*n*=6) and 20.89±0.13 (*n*=6), respectively. These values are in good agreement with the conditional stability constants as found in various

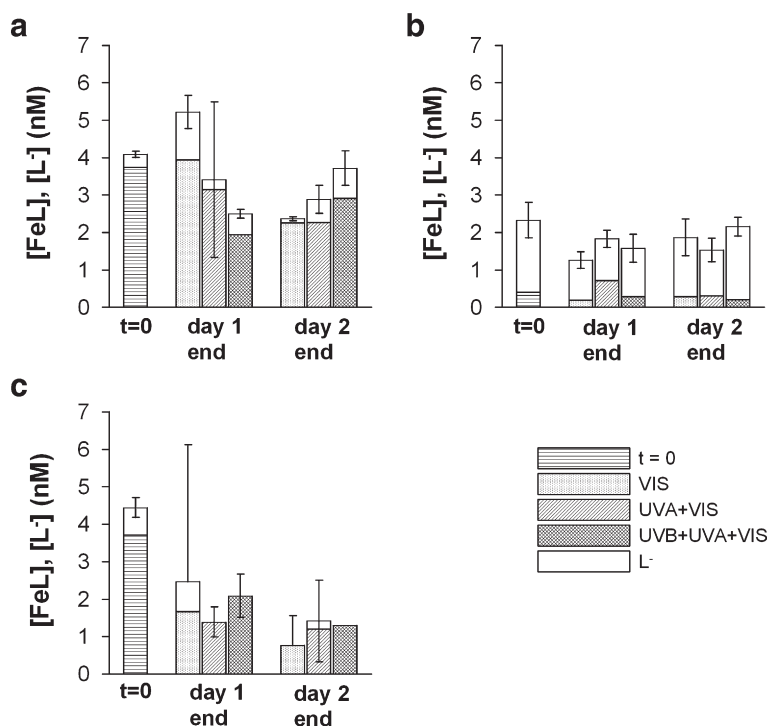


Fig. 6. The concentrations of FeL and excess ligand L in a) experiment A (+5 nmol L⁻¹ Fe, +*Thalassiosira* sp.), b) experiment B (+*Thalassiosira* sp.), and c) experiment C (+5 nmol L⁻¹ Fe, +*C. brevis*). The given errors show the 95% confidence interval.

oceans (Boyé et al., 2005; Gledhill and van den Berg, 1994; Wu and Luther, 1995).

The average total concentration of L in experiment A (containing $5.32 \text{ nmol L}^{-1} \text{ Fe}$) was $3.35 \pm 1.05 \text{ eq nmol L}^{-1} \text{ Fe}$ ($n=7$), upon addition of 5 nM Fe(III) . The ligand concentration in experiment A was roughly twofold higher than the concentration of L in experiment B without Fe(III) addition (mean $1.71 \pm 0.31 \text{ eq nmol L}^{-1} \text{ Fe}$, $n=7$, Fig. 6). The total concentration of L varied with the different light treatments in experiment A. During the first day, the total L concentration in the VIS treatment increased and then decreased during the second day. The total L concentration in the UVB+UVA+VIS treatment decreased during the first day and increased during the second day. The total L concentration in the UVA+VIS treatment decreased by $\sim 0.37 \text{ eq nM Fe}$ from the first to the second day.

In contrast to experiments A and B containing *Thalassiosira* sp., the total concentration of L decreased during all irradiance treatments of experiment C containing *C. brevis* (Fig. 6). Note that no correlations between excess L, and/or FeL, and the different optical treatments were observed in any of the experiments (Fig. 6). Also, no correlations were found between the concentrations of FeL, and/or excess L, and the photoproduction of Fe(II). Experiment C showed an initial decrease in L concentration on the first day, but no further decline on the second day. Furthermore, the ligands in experiment C were almost saturated with Fe (Fig. 6) and contained ligands with lower conditional stability constants compared to experiments A and B. We observed a high TAC-labile Fe concentration in comparison to the concentrations of TAC-labile Fe close to zero in experiments A and B (TAC-labile Fe being the Fe bound by TAC ($10 \text{ } \mu\text{mol L}^{-1}$) after $>12 \text{ h}$ equilibration). In experiment C, this TAC-labile Fe was $\sim 0.4 \text{ nM}$ at $t=0$ but increased during the first day to $\sim 1.5\text{--}2.1 \text{ nmol L}^{-1}$ and remained constant on the second day (Table 5). We have indications that this TAC-labile Fe, observed during titrations, represents Fe bound to a weak ligand class ($\log K' < 18$). Apparently the concentration of this weak ligand class is low since it cannot compete with TAC (Gerringa et al., 2007; Rijkenberg et al., 2006b). The conditional stability constant is an average of the conditional stability constants of a range of organic ligands present in natural seawater. A lower value of $\log K'$ indicates the presence of a relative higher content of weaker ligands. Analysis of the $\log K'$ by analysis of variance (ANOVA) revealed significant differences between the three experiments ($P < 0.001$). Post-hoc analysis using Tukey's method (Montgomery, 1997) showed that the $\log K'$ of experiment C was significantly lower than in experiments A and B ($P < 0.05$).

4. Discussion

On the whole, this study showed an increase in the reactive Fe concentration in the experiments with *Thalassiosira* sp. and *C. brevis*. Furthermore, the results as discussed below suggest that there might be species

dependent differences in the mechanism that increases the reactive Fe concentration in the experiments.

4.1. Total DFe

It is no surprise that there is no relationship between the photoproduction of Fe(II) and the concentration of DFe, because DFe is an operationally defined Fe fraction based on filtration (0.2 or $0.4 \text{ } \mu\text{m}$). DFe consists of different chemical Fe forms including a small colloidal phase as well as inorganic ions and organically complexed Fe (Nishioka et al., 2001; Rue and Bruland, 1995; Wells and Goldberg, 1992). Although organically complexed Fe can be photoreduced (Barbeau et al., 2001), it has been reported that Fe-binding ligands can also be photostable (Rijkenberg et al., 2006b). Overall, it is uncertain if all DFe is available for photoreduction.

4.2. Ferrous iron

The very similar results between the Fe(II) concentration with time of the first day of the abiotic experiment and the experiments A and C suggest the photoreduction of colloidal Fe(III). These Fe colloids might form a mixture of inorganic Fe(III) and organic material. Note that the colloidal fraction is seen as an important candidate to contain a photoreducible Fe fraction (Rijkenberg et al., 2005; Rijkenberg et al., 2004; Wells et al., 1991), and is present in coastal regions as well as in the open ocean (Bergquist et al., 2007; Nishioka et al., 2005).

Thalassiosira sp. (experiments A and B) and *C. brevis* (experiment C) might influence the redox speciation of Fe via different mechanisms. In the incubation with *Thalassiosira* sp. (experiment A) the production of Fe(II) was irradiance dependent as confirmed by the lower Fe(II) concentrations in the dark. Conversely, the Fe(II) concentrations during the dark period in the incubations with *C. brevis* were higher than during the previous irradiance period and stayed stable during the second day of irradiance, although a slight increase in Fe(II) was observed in the bottle receiving UVB+UVA+VIS. This suggests that a light independent process, such as superoxide or membrane reductase mediated Fe(III) reduction, might play an additional role (Salmon et al., 2006).

There are several mechanisms described for the reduction of Fe(III) by microorganisms that are independent of irradiance. Some microorganisms employ an Fe uptake pathway in which Fe(III) is reduced to Fe(II) via superoxide radicals generated by enzymes in the cell membrane (Fujii et al., 2006; Kustka

et al., 2005; Rose et al., 2005; Rose and Waite, 2005). Alternatively, Fe(III) can be reduced by membrane reductases (Allnut and Bonner, 1987; Lynnes et al., 1998; Maldonado and Price, 2000; Weger et al., 2002) and additionally via either dissociative (Shaked et al., 2005) or non-dissociative reduction (Salmon et al., 2006) of Fe complexed to Fe-binding ligands.

In contrast to the abiotic experiment, the Fe(II) concentrations increased in the experiments with *Thalassiosira* sp. (experiments A and B) during the second day. In experiment A, colloidal Fe was the main Fe fraction, whereas in experiment B most of the Fe(III) was organically complexed. However, a small colloidal Fe(III) fraction might be present (Rijkenberg et al., 2006a). These increasing Fe(II) concentrations in experiments A and B might have been the result of more effective Fe(III) photoreduction possibly due to organic substances released by the diatoms (Hudson et al., 1992; Kuma et al., 1995; Kuma et al., 1992; Öztürk et al., 2004; Song et al., 2005). These photosensitive organic components would facilitate a LMCT reaction with colloidal bound Fe (Siffert and Sulzberger, 1991) or with Fe bound by stronger Fe-binding ligands (Kunkely and Vogler, 2001). Furthermore, photoproduced superoxide (Croot et al., 2005; Fujii et al., 2006; Rijkenberg et al., 2006a) or superoxide produced by *Thalassiosira* sp. (Kustka et al., 2005) could be involved in the reduction of amorphous Fe hydroxides. Another possibility is a slower Fe(II) oxidation rate. Shaked et al. (2002) observed slower Fe(II) oxidation rates in waters enriched with algae as compared to abiotic waters of Lake Kinneret. However, simultaneously, Shaked et al. (2002) found increased Fe(III) reduction rates in the presence of the natural phytoplankton assemblage. Croot et al. (2001) explained the retention of Fe(II) during the iron enrichment experiment SOIREE by stabilisation of the Fe(II) by organic complexation. However, although very plausible there is still no direct evidence for organic complexation of Fe(II) in seawater.

Interestingly, apart from an increase in the Fe(II) concentration at the second day in experiments A and B, we also observed that the percentage of Fe(II) produced by UVB+UVA+VIS was almost equal to that produced by UVA+VIS and VIS. This means that VIS is the main contributor to the Fe(II) photoproduction and that UVB and UVA only play minor roles. This is contradictory to what we would expect with colloidal Fe in an abiotic experiment (Rijkenberg et al., 2005; Rijkenberg et al., 2004). We can only speculate on the mechanism behind the increased importance of VIS in the photoproduction of Fe(II). Possible mechanisms directly or indirectly

promoting Fe(II) photoproduction by VIS are: i) the stimulation of biological processes by VIS, e.g. hydrogen peroxide production or Fe(III) reduction by membrane reductases, ii) the UV-mediated photodegradation of the excreted organic constituents or a slower production of organic constituents by phytoplankton due to photoinhibition, and iii) the extension of the absorption band of the Fe(III) towards VIS due to a chemical alteration of the Fe(III) pool by for example the presence of weak Fe complexing organic substances. However, using an analysis of co-variance, with time as the covariate, showed that the irradiance treatment did of excreted organic constituents not significantly affect the slopes of the Fe(II) concentration with time in the experiments A and B (Table 4). When biological processes or UV degradation of excreted organic constituents would play a role we would expect that the slope of Fe(II) production versus time would be affected either by the UV or by differences in cell densities. Cell densities were not the same in the incubations. In experiment A we found 17% more diatoms in the incubations receiving VIS and 10% less diatoms in the incubations receiving UVB+UVA+VIS as compared to the incubations receiving UVA+VIS. In experiment B we found 30% more diatoms in the incubations receiving VIS and 20% less diatoms in the incubations receiving UVB+UVA+VIS as compared to the incubations receiving UVA+VIS. Thus, the similar slopes of the Fe(II) photoproduction with time suggests that an extension of the absorption band of the Fe(III) towards longer wavelength might be the mechanism increasing the importance of VIS. Such an effect has been observed previously with the complexation of Fe by oxalate extending the absorption band of Fe(III) into the visible light (Zuo, 1995). The reason that this effect is only observed on the second day might be

Table 4
Analysis of co-variance with time as the covariate shows that treatment (irradiance spectrum) did not significantly affect the slope of the Fe(II) concentration with time during the second day of experiment A and B

Source	df	S.S. $\times 10^{-2}$	MS	F	P
<i>Experiment A</i>					
Time	1	13.7	13.7	48.8	0.001
Treatment	2	0.5	0.26	0.2	0.459
Time*treatment	2	0.6	0.32	1.1	0.396
Error	5	1.4	0.28		
<i>Experiment B</i>					
Time	35.5	1	35.5	5.6	0.077
Treatment	16.2	2	8.1	1.3	0.371
Time*treatment	15.8	2	7.9	1.3	0.379
Error	25.3	4	6.3		

* denotes a significant result $P \leq 0.001$.

that oxidation of photoproducted Fe(II) induces the formation of fresh colloidal Fe which incorporates the organic substances that are released by phytoplankton, and thus leads to a higher photoproduction of Fe(II). Only at day 2 the Fe(II) production exceeded the decreasing concentration of photoreducible Fe from the amorphous Fe oxyhydroxides. Another explanation is that *Thalassiosira* sp. only started to produce these photosensitive organic substances after the first day. Although, we see a similar effect in experiment C this could also partly be due to irradiance independent processes that play a role in the Fe(II) production in the incubations with *C. brevis*.

A side effect of the extension of the absorption band of the Fe(III) species towards VIS would be an enhancement in the photoreduction of Fe(III) in the euphotic zone. This because the longer wavelengths of VIS attenuate less fast than the shorter wavelengths of UV (Boucher and Prezelin, 1996).

Overall, these processes induced by the presence of diatoms that increased the Fe(II) production could play an important role in the retention and transformation of Fe from biological and lithogenic particulate Fe(III) in the dissolved phase (Boyd et al., 2005).

4.3. Organically complexed ferric iron

The ligand concentration in experiment A after addition of the diatoms is roughly double the concentration of Fe-binding ligands as measured in experiment B. Although it is suggested that *T. weissflogii* produces Fe-binding ligands (Fuse et al., 1993), it is unlikely the *Thalassiosira* sp. in experiment A produced twice the concentration of Fe-binding ligands as in experiment B immediately after their addition to the seawater ($t=0$). Note that in both experiments we used *Thalassiosira* sp. starting with ~ 50 cells/mL, and the only difference is the addition of 5 nM Fe(III) in experiment A. The 5 nM Fe(III) would lead to saturation of the empty organic ligand sites and next formation of amorphous Fe oxyhydroxides (Rijkenberg et al., 2006a). A general assumption used in calculating the dissolved FeL concentration is that all DFe is organically complexed. This assumption is based on the finding that 99% of the DFe concentration in seawater is organically complexed (van den Berg, 1995). Using size fractionation we know now that the DFe phase also contains small colloidal Fe (Nishioka et al., 2005; Wells, 2003). However, the conditional stability (kinetics of formation and dissociation) of the amorphous Fe colloids should equal the conditional stability of the binding by organic Fe-binding ligands in order to exist in the presence of free

strong Fe-binding ligands. An increased apparent Fe-binding capacity could be caused by a mixture of inorganic colloids and organic forms (Boyé et al., 2005; Croot and Johansson, 2000). The colloidal Fe(III) oxyhydroxides resulting from the Fe addition in experiment A could have formed surface-active amorphous colloids that appeared as a (saturated) strong Fe-binding capacity upon titration. Thus the addition of 5 nM Fe(III) to experiment A induced the formation of amorphous Fe oxyhydroxides with an Fe-binding capacity equal to the organic Fe-binding ligands present. Geringa et al. (2007) reported formation and dissociation rate constants for the binding of iron to colloidal iron resulting in conditional stability constants ($\log K' = k_f/k_d$, with respect to $[\text{Fe}^{3+}]$) between 10^{18} and 10^{19} . Boyé et al. (2005) found that most of the Fe-binding capacity in pre-Fe infusion conditions during EisenEx were in the truly soluble form (<200 kDa). We assume that the Fe-binding capacity in experiment B, where no Fe(III) was added, is due to organic Fe-binding ligands.

In experiment A the change in the concentration of strong Fe-binding ligands varied with irradiance treatment (Fig. 6a). A variation in Fe-binding capacity might be the result of changes in the surface-active Fe-binding amorphous colloids, induced by the irradiance treatment, and possibly by the diatoms. However, we cannot exclude the possibility that *Thalassiosira* sp. produced Fe-binding ligands (Fuse et al., 1993) although this was not measured in experiment B (Fig. 6b).

The appearance of a TAC-labile Fe concentration suggested the presence of weak Fe-binding ligands in experiment C with *C. brevis*. It is uncertain if these weak Fe-binding ligands had a biological origin or that they originated from a structural/chemical alteration of the strong Fe-binding ligands. In each bottle, the increased TAC-labile Fe concentration coincided with a decreased concentration of the strong Fe-binding ligand L with respect to its original concentration strong Fe-binding ligand L at $t=0$ ($[L]_{t=0} - [L]_{t=x}$). The linear relationship of [TAC-labile Fe] with respect to $[L]_{t=0} - [L]_{t=x}$ was very good with a R^2 of 0.95 (Table 5). However, note that the determination of $[L]_{t=x}$ is not fully independent from the TAC-labile Fe concentration, furthermore we do not refer here to a correlation between the TAC-labile concentration and the strong Fe-binding capacity in time but in relation the different culture bottles. The relationship between the TAC-labile Fe concentration and $[L]_{t=0} - [L]_{t=x}$ might imply that biologically induced changes in the ligands from the stronger ligand class resulted in the presence of a weaker ligand class.

Table 5

The TAC-labile Fe concentration (TAC-labile Fe being the Fe bound by TAC ($10 \mu\text{mol L}^{-1}$) after >12 h equilibration) and the decrease in the concentration of strong Fe-binding ligand with respect to its original concentration strong Fe-binding ligand L at $t=0$ ($[L]_{t=0} - [L]_{t=x}$)

Sample	TAC-labile Fe (nM)	$[L]_{t=0} - [L]_{t=x}$ (nM)
$t=0$	0.4	0
<i>End day 1</i>		
VIS ^a	2.13	1.98
UVA + VIS	1.6	3.06
UVB + UVA + VIS	1.56	2.36
<i>End day 2</i>		
VIS	2.15	3.68
UVA + VIS	1.56	3.03
UVB + UVA + VIS	1.87	3.14

There is a strong linear correlation between TAC-labile Fe (y -axis) and $[L]_{t=0} - [L]_{t=x}$ (x -axis) ($y=0.45x+0.38$, $R^2=0.95$), but note that the determination of $[L]_{t=x}$ is not fully independent from the TAC-labile Fe concentration.

^a This datapoint was excluded from the linear relationship between TAC-labile Fe and $[L]_{t=0} - [L]_{t=x}$ because of its high error in the measurement of L_{total} (see Fig. 6c day 1).

In experiment C, the concentration of TAC-labile Fe observed was low at $t=0$, and did not exist at all in the experiments A and B. The presence of the weaker Fe-binding ligands as indicated by the TAC-labile Fe concentration (Gerringa et al., 2007; Rijkenberg et al., 2006b) resulted in a significantly lower log K' of the Fe-binding ligands in experiment C as compared to the log K' of the Fe-binding ligands in the experiments A and B. Similar high TAC-labile Fe concentrations have been reported during bloom situations (Croot and Johansson, 2000; Rijkenberg et al., 2006b) and could be the result of uptake of organically complexed Fe. It might be that processes such as superoxide reduction and reductase mediated Fe reduction (Maldonado and Price, 2001; Rose and Waite, 2005; Salmon et al., 2006) are responsible for both the irradiance independent high Fe(II) concentrations as well as the increased concentration of TAC-labile Fe in the incubations with *C. brevis*. Overall, this modification of the Fe into a more reactive Fe species is ascribed to the presence of *C. brevis*. As a result the Fe(III) is more easily available in the context of its necessary dissociation from the ligand for uptake of Fe into the diatom cell.

5. Conclusion

Our results show that the presence of the diatoms may be important for the speciation of Fe, both for $[\text{FeL}]$ and the photoproduction of Fe(II). The Fe(II) concentration on the first experimental day was wavelength-

dependent when 5 nM Fe was added (exp. A and C), although on the second day this wavelength-dependency disappeared. On the second day of the incubations with *Thalassiosira* sp. the increased photoproduction of Fe(II) appeared to coincide with an increase in the relative importance of VIS, suggesting a chemical alteration of the photoreducible Fe(III) pool. The release of carbohydrates and/or other organic compounds resulting in enhanced Fe photoreduction (Hudson et al., 1992; Kuma et al., 1992) in the visible part of the spectrum would result in an increase in the reduction and dissolution of Fe from colloidal material and subsequently increase the Fe available for biological uptake (Johnson et al., 1994; Wells and Mayer, 1991). The reduction of Fe(III) in incubations with *C. brevis* may have included an irradiance independent process like that of superoxide or reductase mediated Fe(III) reduction.

The enhanced TAC-labile Fe concentration occurred only in the experiment with *C. brevis* suggesting that the presence of *C. brevis* resulted in the introduction of a weaker class of ligands. An increase in TAC-labile Fe concentration means an increase in Fe more kinetically labile with respect to ligand exchange, i.e. subsequently improving its biological availability (Morel et al., 1991). Overall, we found in the experiments with *Thalassiosira* sp. as well as *C. brevis* an increase in the reactive Fe pool.

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